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Intrinsic Toxicity of Antibodies to the Globular Domain of the Prion Protein

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To the editor: While making their case for the immunotherapy of prion diseases, Purro *et al* (1) suggest that the toxicity of antibodies against the globular domain of the prion protein, which was observed in numerous studies, may be unspecific. The published literature, however, tells a different story (2-9). Firstly, toxicity was never observed in mice devoid of the prion protein – a finding impossible to reconcile with the alleged lack of antibody specificity. Secondly, toxicity was reproducibly blocked by preincubation of antibodies with recombinant prion protein, indicating the requirement for a reactive paratope. Thirdly, toxicity was reproduced with single-chain nanobodies directed against PrP, thereby excluding any role for complement fixation or other effector functions of antibodies. Fourthly, no toxic effects were observed with unrelated antibodies (including pooled IgG, anti-NOGO receptor and BRIC222) administered in similar paradigms. Fifthly, antiprion antibody toxicity was commensurate to the level of PrP expression, with overexpression leading to exaggerated toxicity. Sixthly, toxicity required PrP expression by neurons. Finally, toxicity was only seen in mice whose PrP protein contained an intact amino

terminus. Assuming that Purro *et al* (1). adhere to the conventional meaning of “specificity” in an immunological context (i.e. selectivity of the paratope-epitope interaction), one can safely conclude that said specificity is confirmed by a vast body of experimental evidence.

Furthermore, Purro *et al* (1) incorrectly state that antibody toxicity was achieved only at micromolar concentrations. Toxicity in cerebellar organotypic cultured slices (COCS), as assessed by the disappearance of the NeuN neuronal marker (indicative of neuronal death within the internal granule cell layer), is induced by antibody POM1 at a concentration of 270 nanomoles/liter in COCS expressing wild-type levels of PrP (4). Besides, a single exposure to 33 nanomolar ICSM18 or POM1 is sufficient to induce spontaneous currents (a surrogate of neurotoxicity) in N2a cells expressing normal levels of wild-type PrP^{C} (7). Another PrP ligand discussed by the authors, Aβ, suppresses long-term potentiation in hippocampal slices at 0.5-1 μM concentrations (10-12) which are similar to (or higher than) those of toxic anti-PrP antibodies. Therefore, there is no factual basis to the dismissal of antibody toxicity as unspecific effects due to excessive concentrations.

Purro *et al* (1) claim that no toxicity was seen after intrahippocampal injection of up to 2 μg of various anti-PrP antibodies. Again, this is not in line with the published evidence. Solforosi et al. described neurodegeneration upon stereotactic injection of 2 μg of antibodies P and D13 (3), and we reproduced neurodegeneration with D13 both in COCS and upon stereotactic injection (4, 6). We are aware that Klöhn and Collinge failed to confirm these findings (13), yet negative data can be caused by many confounders and should only be considered informative if the underlying mechanism is elucidated.

Purro *et al* (1) incorrectly characterize the neurotoxicity described in our stereotaxic injection studies as “apoptosis at the cannula site” - a description presumably meant to imply mechanic needle damage. In reality, we reported that injection of 6 μg of antibody ICSM18 induced lesions of up to 1.4 mm^3 (avg±SD: 0.26 ± 0.15 mm^3) in wild-type female mice, which is fourteen times the

largest lesion (0.1 mm^3) seen after control injection of 6 µg BRIC222 (avg±SD: 0.06 ± 0.05 mm^3) (6). What is more, chronic administration of antibodies through osmotic minipumps to *tga20* mice overexpressing PrP (14) resulted in massive destruction of up to 13% (avg±SD; 9±3%) of the total brain volume (Fig. 1 and ref. (6)).

Purro *et al* (1) refer to an effective dose with no adverse effects of 1 µg/h for antibody 31C6 (total dose 336 µg, 14 days (15)) and claim a therapeutic range of 2 logs. This alleged lack of toxicity may not be fully informative since 31C6 does not interact with helix-3 of PrP (16). While we did not have the opportunity to study antibody 31C6, we found that chronic administration of an equimolar amount of POM1 induces massive tissue damage in *tga20* mice (Fig. 1) as early as four days (cumulative dose: 14.4 µg POM1 scFv) after minipump implantation (diffusion-weighted magnetic resonance imaging, Figure 4 in (6)). One can only hope that such dramatic adverse effects will remain confined to PrP-overexpressing mice.

Purro *et al* (1) note that Klyubin et al. failed to detect neurotoxicity upon intracerebroventricular administration of 30 µg and intracardiac administration of 6mg of antibody PRN100 (17). While this is encouraging, their analysis was limited to histology 4 hours after intracerebroventricular injection and 14 hours after intracardiac administration. At this early time point, only minimal lesions are detectable by magnetic resonance imaging, arguably the most sensitive indicator of damage (Figure 4 in ref. (4)), and histology revealed early signs of neurodegeneration only in *tga20* mice challenged intracerebrally with 6 µg antibody (Supplementary Figure 13 in ref. (4)). Purro *et al* (1) mention a single ascending dose-finding study of PRN100 in cynomolgus monkey at intravenous doses up to 200 mg/kg allegedly described by Klyubin et al (17). However, the actual data are not shown in the cited publication and do not seem to be publicly available.

In summary, we commend and share the enthusiasm with which Purro et al. promote antibody therapies against prions. Like them, we feel for the patients affected by these terrible diseases. However, the interests of patients are best served by reading and correctly interpreting the

available literature, and by addressing any safety flags dispassionately, collaboratively, and without preconceptions.

Figure Legends

Figure 1: Gross brain destruction induced by chronic exposure to antibody POM1. Representative micrographs of *tga20* mouse brains (immunostaining: glial fibrillary acidic protein) 21 days after the implantation of a mini-osmotic Alzet pump delivering a single-chain variable fragment (scFv) of POM1 (0.15 µg/h, cumulative dose of 75 µg) intracerebroventricularly. Gross damage to the brain architecture with conspicuous hydrocephalus e vacuo indicative of tissue loss. Note the massive astrogliosis extending to the contralateral hemisphere. In contrast, no lesion was detected in *Prnp*$^{o/o}$ mice. Adapted from Reimann et al (6).

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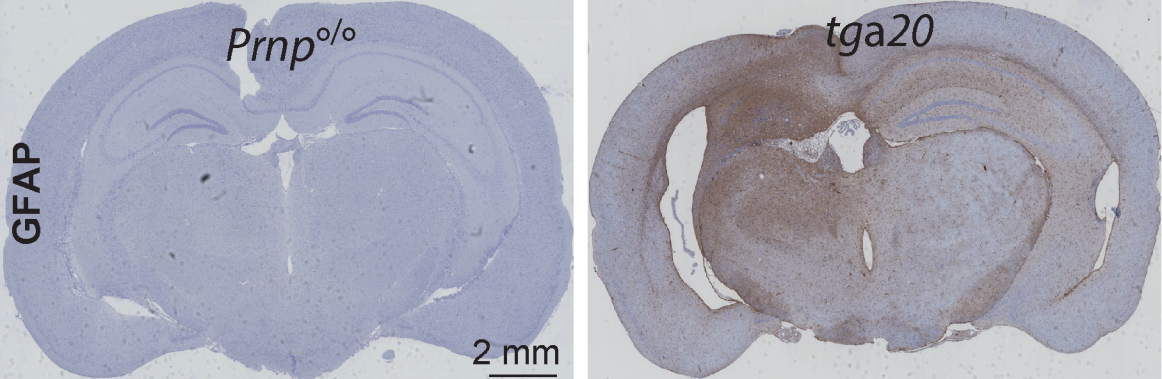
Prnp^o/o
GFAP
2 mm
tga20